

## TABLE OF CONTENTS

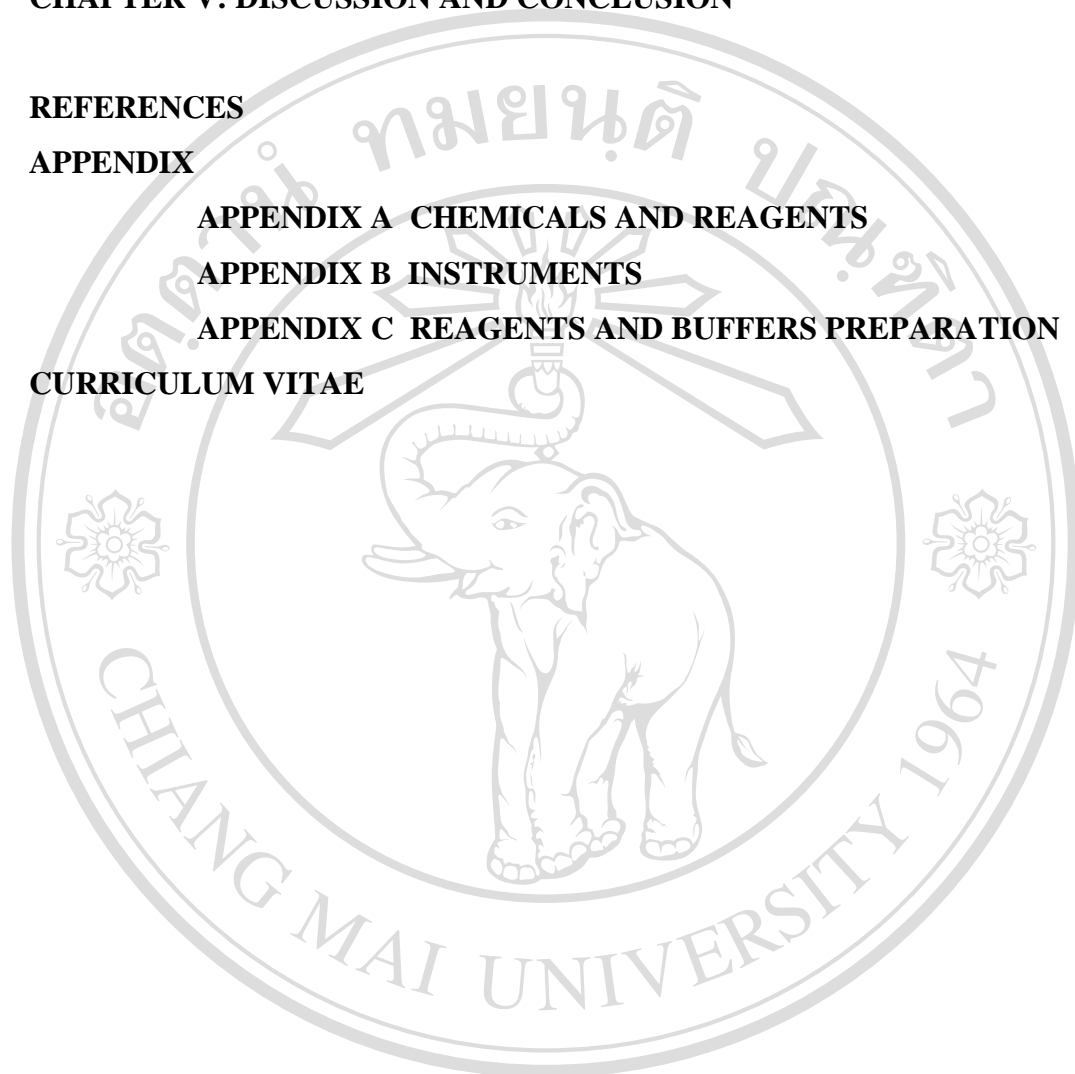
	PAGE
<b>ACKNOWLEDGEMENT</b>	iii
<b>ABSTRACT</b>	v
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xv
<b>ABBREVIATIONS</b>	xvi
<b>CHAPTER I: INTRODUCTION</b>	1
<b>CHAPTER II: LITERATURE REVIEWS</b>	4
1. Biology of Human papillomavirus (HPV)	5
1.1 Classification	5
1.2 Virion structure	5
1.3 Viral genome structure and organization	7
1.4 Viral gene products and their biological functions	8
1.4.1 E1 protein	8
1.4.2 E2 protein	8
1.4.3 E4 protein	8
1.4.4 E5 protein	10
1.4.5 E6 protein	10
1.4.6 E7 protein	11
1.4.7 E8 <sup>E2</sup> protein	12
1.4.8 L1 and L2 proteins	12
1.5 Viral replication	12
1.6 Pathogenesis of HPV infection	15
1.7 Clinical manifestations	16
2. Cervical cancer	19

2.1	Epidemiology	19
2.2	Pathogenesis of cervical cancer	20
2.3	Cervical precancerous lesions	20
2.4	Risk factors and cofactors in cervical cancer development	22
2.4.1	HPV risk factors	22
2.4.1.1	High-risk HPV types	22
2.4.1.2	Viral variants	22
2.4.1.3	Viral load	23
2.4.1.4	Viral integration	23
2.4.1.5	Multiple HPV co-infections	23
2.4.2	Non-HPV risk factors	23
2.4.2.1	Long-term use of oral contraceptives (OCs)	24
2.4.2.2	High parity	24
2.4.2.3	Smoking	24
2.4.2.4	Other sexually transmitted infections (STIs)	25
2.4.2.5	Life style	25
2.4.2.6	Immunosuppression	25
2.4.2.7	Genetic predisposition	25
3.	HPV immunology and vaccines	26
3.1	Innate immunity	26
3.2	Humoral immunity	26
3.3	Cellular immunity	27
3.4	HPV vaccines	27
3.4.1	Prophylactic vaccines	27
3.4.2	Therapeutic vaccines	28
4.	Cervical cancer screening	28
4.1	Pap smear screening	28
4.2	Visual inspection with acetic acid (VIA)	29
4.3	HPV DNA detection	30
4.3.1	Hybridization-based assay	30

4.3.2	Polymerase chain reaction-based assays	31
4.3.2.1	Type-specific PCR versus broad-spectrum PCR	31
4.3.2.2	Real-time PCR	34
4.3.3	HPV genotyping analysis	37
4.3.3.1	PCR and restriction enzyme analysis (PCR-REA)	37
4.3.3.2	Direct DNA sequencing analysis of PCR products	38
4.3.3.3	Microtiter plate hybridization analysis	39
4.3.3.4	Reverse hybridization analysis	39
<b>CHAPTER III: RESEARCH DESIGN, MATERIALS AND METHODS</b>		41
1.	Research design	41
2.	Materials and methods	42
2.1	Extraction of recombinant plasmids containing HPV DNA type 6, 11, 16, 18, 31, 33, and 35 DNA from the transformed <i>E. coli</i>	42
2.2	Designation, selection, and determination of the optimal conditions of TaqMan-based real-time PCR	43
2.2.1	Primers, probe designation and selection	43
2.2.2	Determination of the optimal conditions of TaqMan-based Real-time PCR	45
2.2.2.1	Determination of the optimal annealing temperature of primers and probe	45
2.2.2.2	Determination of the optimal concentration of primers and probe	46
2.3	Determination of the sensitivity of TaqMan-based real-time PCR for detection of HPV DNA	46
2.4	Detection of HPV DNA in cervical specimens by using TaqMan-based real-time PCR	47
2.4.1	Specimen collection and storage	47
2.4.2	DNA extraction from cervical cells preserved in	

<i>Liqui-PREP™</i> solution	47
2.4.2.1 Determination the optimal concentration of Proteinase K and incubation time for DNA extraction method	47
2.4.3 DNA extraction from cervical specimens	49
2.4.4 Detection of HPV DNA by using TaqMan-based real-time PCR	49
2.5 Detection of HPV genotype by restriction enzyme analysis (REA)	50
2.5.1 Restriction enzyme selection	51
2.5.2 HPV genotyping by REA	51
2.5.2.1 Amplification of HPV DNA positive samples by using conventional PCR	51
2.5.2.2 Purification of the PCR product	52
2.5.2.3 HPV Genotyping by REA	52
<b>CHAPTER IV: RESULTS</b>	54
1. Extraction of recombinant plasmids containing HPV type 6, 11, 16, 18, 31, 33, and 35 DNA from the transformed <i>E. coli</i>	54
2. Determination the optimal conditions of TaqMan-based real-time PCR	55
2.1 Determination the optimal temperature of primers and probe	55
2.2 Determination the optimal concentration of primers and probe	56
3. Determination of the sensitivity of TaqMan-based real-time PCR in detection of HPV DNA	58
4. Detection of HPV DNA in cervical specimens by using TaqMan-based real-time PCR	59
4.1 Determination the optimal concentration of Proteinase K and incubation time for DNA extraction method	59
4.2 Detection of HPV DNA by using TaqMan-based real-time PCR	62
5. Detection of HPV genotypes by restriction enzyme analysis (REA)	63

<b>CHAPTER V: DISCUSSION AND CONCLUSION</b>	<b>65</b>
<b>REFERENCES</b>	<b>68</b>
<b>APPENDIX</b>	<b>79</b>
<b>APPENDIX A CHEMICALS AND REAGENTS</b>	<b>80</b>
<b>APPENDIX B INSTRUMENTS</b>	<b>82</b>
<b>APPENDIX C REAGENTS AND BUFFERS PREPARATION</b>	<b>83</b>
<b>CURRICULUM VITAE</b>	<b>88</b>



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
1. Size and function of papillomavirus proteins	13
2. Clinical associations of HPV genotypes	17
3. The ratio of concentrations between forward primers and reverse primer used in TaqMan-based real-time PCR	46
4. The varying concentration of proteinase K and incubation time used for extraction of DNA from preserved cervical cells	48
5. The L1 REA genotyping patterns constructed according to the reference HPV sequences obtained from Genbank	53
6. Demonstrate the results of quantity and quality of HPV recombinant plasmids preparations	55
7. The $C_t$ value from real-time PCR performing with gradient temperature between 50-60°C	56
8. Results of $C_t$ value obtained from PCR assay at different concentration of PGMY09/11 A-E (forward) primers with fixed concentration (400 nM) of GP6+ reverse primer	57
9. The results in $C_t$ value obtained from each probe concentrations	58
10. The results in $C_t$ value of the sensitivity determination of TaqMan-based real-time PCR in detection of plasmid HPV16 DNA	59
11. Comparison between TaqMan-based real-time PCR and visual inspection with acetic acid (VIA) in detection of HPV DNA from cervical cell scrapes	62
12. Genotype distribution of HPV as determined by PCR-REA	63

## LIST OF FIGURES

<b>Figure</b>	<b>Page</b>
1. Electron micrograph of HPV1 virion particles (55 nm in diameter)	5
2. Surface shaded and sectioned displays of the HPV1 reconstructions viewed along a two-fold axis of symmetry	5
3. Organization of the HPV16 genome	8
4. Cellular interactions of E6 and E7 oncoproteins and their synergy in induction of cell immortalization	12
5. Productive life cycle of HPV	14
6. HPV-mediated progression to cervical cancer	16
7. Natural history of cervical cancer development	19
8. Progression from a benign cervical lesion to invasive cervical cancer	20
9. Outline of the HPV-DNA genome, presented in a linear form	32
10. Fluorogenic mechanisms	34
11. Amplicon detection by 5' Nuclease oligoprobes	35
12. Outline and example of the reverse hybridization HPV line probe assay	40
13. The location of PGMY11-A to E forward primer, Modified GP6+ reverse primer and Modified GP5+ HPV Probe on L1 gene	44
14. The amplification of the plasmid HPV DNA fragments from colonies of transformed <i>E. coli</i> by PCR	54
15. Illustrations the agarose gel electrophoresis of PCR amplification products of $\beta$ -globin gene extracted from cervical samples using different concentrations of proteinase K and lyses time	61
16. The illustration of PCR-REA patterns of HPV genotypes from cervical cell scrapes	64

## ABBREVIATIONS

%	percent
°C	degree Celsius
β	beta
μg	microgram
μl	microliter
μM	micromolar
Å	angstrom
A	adenine
aa	amino acid
ag	attogram
ASC-US	atypical squamous cell of undetermined significance
ASR	age-standardized incidence rate
BGL	beta-globin
BHQ	black hole quencher
bp	base pair
C	cytosine
CIN	cervical intraepithelial neoplasia
CKI	cyclin-dependent kinase inhibitor
C <sub>t</sub>	threshold cycle
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanine triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dsDNA	double-stranded deoxyribonucleic acid

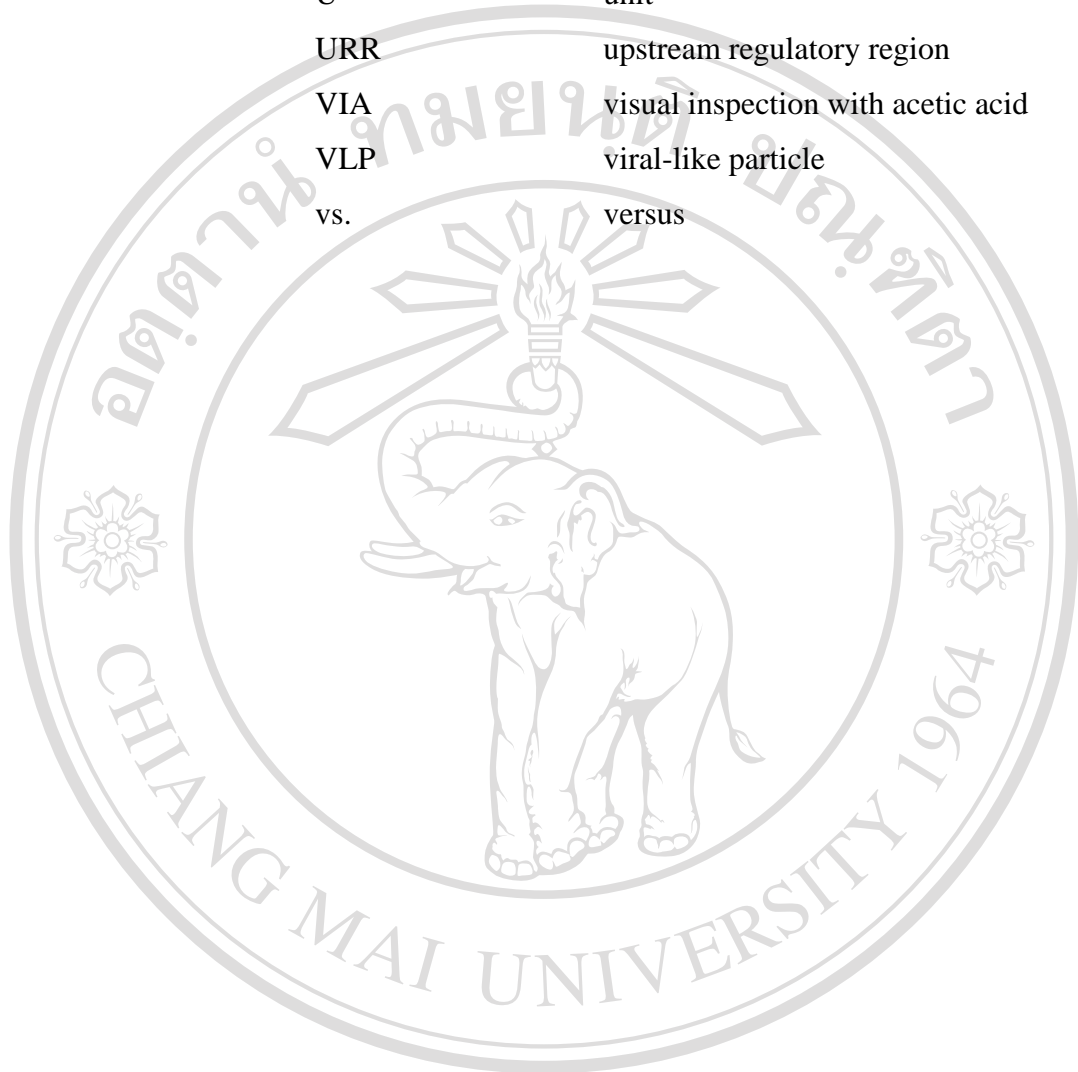
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright © by Chiang Mai University  
 All rights reserved



dTTP	deoxythymidine triphosphate
dUTP	deoxyuridine triphosphate
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylenediaminetetraacetic acid
e.g.	exempli gratia (for example)
EGF	epidermal growth factor
E6-AP	E6-associated protein
EV	epidermodysplasia verruciformis
FAM	6-carboxyfluorescein
FDA	Food and Drug Administration
fg	femtogram
FRET	fluorescence resonance energy transfer
G	guanine
g	gram or gravity
g/mL	gram per milliliter
HC	Hybrid Capture
HLA	human leukocyte antigen
HPV	human papillomavirus
IARC	International Agency for Research on Cancer
kb	kilobase
kDa	kilodalton
l	liter
LB	Luria-Bertani
LBC	liquid-based cytology
LCR	long control region
LiPA	line probe assay
LSIL	low-grade squamous intraepithelial lesion
M	molarity

<i>Mae</i>	<i>Methanococcus aeolicus</i>
mg	milligram
ml	milliliter
mM	millimolar
<i>Mse</i>	<i>Micrococcus species</i>
MW	molecular weight
ng	nanogram
nm	nanometer
nM	nanomolar
NT	non-typable
O.D.	optical density
ORF	open reading frame
<i>ori</i>	origin of replication
Pap smear	Papanicolaou smear
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
pg	pictogram
pRb	retinoblastoma protein
REA	restriction enzyme analysis
RNA	ribonucleic acid
rpm	revolutions per minute
<i>Rsa</i>	<i>Rhodopseudomonas sphaeroides</i>
SD	standard deviation
<i>Sfc</i>	<i>Streptococcus faecium</i>
SSC	squamous cell carcinoma
STIs	sexually transmitted infections
T	thymine
TBE	tris-borate EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>

U	unit
URR	upstream regulatory region
VIA	visual inspection with acetic acid
VLP	viral-like particle
vs.	versus



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved